ON THE ROLE OF ADENOSINE TRIPHOSPHATE

IN ENERGY-MEDIATION

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August 29th, 1974.

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ABSTRACT

ATP and the products of its hydrolysis play a major role in energy conversion which is not shared by other similar biological molecules. Possible explanations for the preference of ATP ~~is~~ are discussed in terms of possible advantages for this choice, and the possible involvement of historical and evolutionary imperatives.

INTRODUCTION

The extensive success of the theories originating with Darwin concerning evolution extends to microscopic and biochemical levels as well as to easily-observable macroscopic development. The law of the survival of the fittest appears to have pertinency for metabolic pathways, proteins, and cell structure, as well as the shape of feet and wings. Plants and animals with inefficient biochemical processes have been selected against and increasingly more efficient metabolic pathways have slowly developed in those living beings which have survived.

The conversion of extraneous energy sources into metabolizable energy is one of the living cell's essential tasks. It would therefore stand to reason that evolution has led to more and more efficient ways of absorbing, storing and converting energy. The development of more efficient enzymatic systems in trapping molecular oxygen for respiration in animals higher on the scale of evolutionary development is a fine example.

While large differences exist in various life forms in many enzymes and biochemical systems, certain small biological molecules (ie; molecules synthesized from precursors in the cell) are still found in all cells. Others, such as steroids, have undergone evolutionary development from common precursors to more specific molecules.

ATP (adenosine triphosphate) is a molecule without which no cell can survive. It is one of the precursors in the biosynthesis of nucleic acids and is thus a sine qua non of the genetic apparatus. The premise that ATP evolved separately in different organisms as a result of similar needs is as unlikely as the proposition that DNA was also the macromolecular product of convergent evolution, in separate organisms. We can conclude that ATP has been a component of cellular systems since the inception of the present genetic apparatus.

ATP also has a direct and no less essential task in the conversion of chemical energy. Though a number of other molecules are also involved in the transfer and storage of energy through the synthesis and cleavage of phosphate bonds, the participation of ATP is by far the most direct and the most extensive. Why this is the case, is by no means self-evident. The

other mononucleotide triphosphates which are precursors of nucleic acids are as prevalent and as essential as ATP, are similar in structure and in physical and chemical properties to ATP. And yet, their participation in energy transfer, while of regulatory significance, is of much smaller proportions.

What factors determined the extensive role of ATP in energetic metabolic processes? Does ATP possess some esoteric quality lacking in what appear as similar molecules? If it does, then what could it be? If not, why has their been no evolutionary progress towards other equally effective, or perhaps superior, molecular mediators?

The factor determines the ~~extensive~~ role of ATP in energetic metabolic processes should be its chemical composition, namely, the structure of the ATP molecule itself.

THE EXTENT OF ATP PARTICIPATION IN ENERGY CONVERSION

ATP is one of a number of molecules which store and transfer energy through phosphate bond synthesis and hydrolysis. Some, such as polyphosphate and phosphocreatine are not universally present yet play an important role in specialized cells. Others are intermediates in such basic metabolic pathways as glycolysis. What experimental evidence supports the extent of direct involvement of ATP?

Respiration and photosynthesis are the major energy-yielding cellular processes. Both are known to occur in membrane-bound complexes; both processes couple chemical energy to oxidation-reduction energy in an enzyme complex known as ATPase where synthesis of an ester of phosphate takes place. Most in-vitro and semi-in-vitro experiments with membrane-bound ATPases of mitochondria (1,2,3), chloroplasts (4) and bacteria (5) show varying degrees of preference for ATP as a substrate (see fig. 1), → should be Table 1 for hydrolysis (the reverse coupling reaction) and synthesis of ATP from ADP. * As well, ATP is the net product of glycolysis (energy-wise).

Secondly, most of the energy-requiring and energy-producing reactions in the cytoplasm show a specificity for ATP (or for producing ATP). Exceptions, such as the substrate phosphorylation of GDP by succinyl-CoA, specificity for CTP in the biosynthesis of phosphoglycerides, and binding of amino-acyl tRNA to the 70S ribosomal comples (GTP requiring) do exist, but constitute exceptions to the rule. What these exceptions do indicate, is that other mononucleotide di- and tri-phosphates can participate in energy mediation. When GTP is produced, however, it rapidly transfers its terminal phosphate group to ADP, in a reaction catalyzed by nucleoside diphosphokinase.

*Studies on a purple, non-sulphur bacteria, Rhodospirillum rubrum, have shown that a light-induced formation of pyrophosphate from orthophosphate can be demonstrated to occur in the chromatophores (6), independent of adenosine phosphate participation (7). This exception will be discussed later.

	SUBSTRATE	Inorganic phosphate released (μmoles/mg.chl. x hr.) Light-dark	Dark
I	ATP	2.83	1.17
	GTP	1.88	0.30
	ITP	1.28	0.30
	ADP	0.00	0.52
	GDP	0.10	0.60
	CDP	0.00	0.45
	UDP	0.10	0.55
II	ATP	2.9	3.2
	GTP	1.1	0.0
	ITP	0.65	1.2
	ADP	0.0	3.0
	GDP	0.0	0.75
	CDP	0.0	0.50
	UDP	0.0	1.57

		^{32}Pi incorporated into NTP (μmoles/mg. chl. x hr.)
III	ADP	1617.0
	GDP	1142.5
	IDP	1225.8
	UDP	213.1
	CDP	00.0

Table 1

Figure 1 - Specificity of ATPase. The specificity of light-triggered and light-dependent ATPase extracted in chloroplast fragments by EDTA was measured by Bennun and Avron (4). I gives values for the hydrolysis of the various substrates by light-dependent ATPase when transferred from the light to the dark, and in the dark. II shows the values obtained for light-triggered ATPase, under the same conditions. III compares the ability of various mononucleotide diphosphates to act as substrates for triphosphate synthesis, in intact, washed chloroplasts. It should be kept in mind that while in these experiments the concentration of substrates is equal, in the viable cell, ATP and ADP have higher relative concentrations which would increase their in-vivo participation in these reactions.

The final observation, which leads us to ascribe a major role to ATP, is that its intracellular concentration is generally higher than those of the other nucleotides (8,9).

We conclude that ATP and the products of its hydrolysis do play the main role in the conversion and transfer of energy in the form of the phosphate bond. ATP is the major (and in many cases likely the sole) product of coupling to electron transport, and the net high-energy product of glycolysis. Its high-energy bonds (ADP is sometimes cleaved to AMP, as well) charge other carriers and enable otherwise energetically-unfavourable reactions to take place by coupling those reactions to hydrolysis of ~~the phosphate bond~~ ATP to ADP, and occasionally AMP + pyrophosphate.

PHYSICAL CHEMICAL ASPECTS OF ATP

The free energy which is released in the hydrolysis of the phosphate bond depends greatly on what moiety the phosphate is linked to before its hydrolysis. When compared with other molecules in the cell with terminal phosphate bonds, the free energy of hydrolysis of ATP to ADP is in the middle. Phosphoenol pyruvate gives a $\Delta G' = 12.8$ Kcal./mole, at the top end of the scale (energy released is greater), 3-Phosphoglyceric acid at the bottom has a standard free energy of hydrolysis of 2.3 Kcal./mole, while for ATP, $\Delta G' = 7.0$ (10). Thus as far as energy transfer is concerned, ATP can be thought of as as a mediator of phosphate bond energy between between compounds of higher energy and those whose phosphate bond is of a lower energy. On the other hand, the equilibrium constants for hydrolysis (and thus the free energy of hydrolysis) of the various other biologically-occurring mononucleotide triphosphates are very similar to those of ATP.

Another important property is the formation of complexes with divalent cations and water, in aqueous solution. Within the cell, ATP is found complexed usually to magnesium and a few water molecules (11). A postulated model for this complex is shown in figure II. The stability constants for various nucleotides with some divalent cations are compared in figure III, from the results of Eva Walaas (12), based on the use of anion exchange. It can be seen that among the triphosphates ~~and among the diphosphates~~ affinity for the individual metal ions is similar, especially for the Mg^{2+} complex.

One last aspect, which will be referred to in the discussion, is the similarity between ATP and ADP, and the dissimilarity between ADP and AMP. Whereas, for example the pK of hydrolysis of ADP to AMP is equal to that of ATP to ADP, the ~~first~~ primary phosphate bond is stronger and the pK and standard free energy of hydrolysis ~~lower~~ of AMP to adenosine lower.

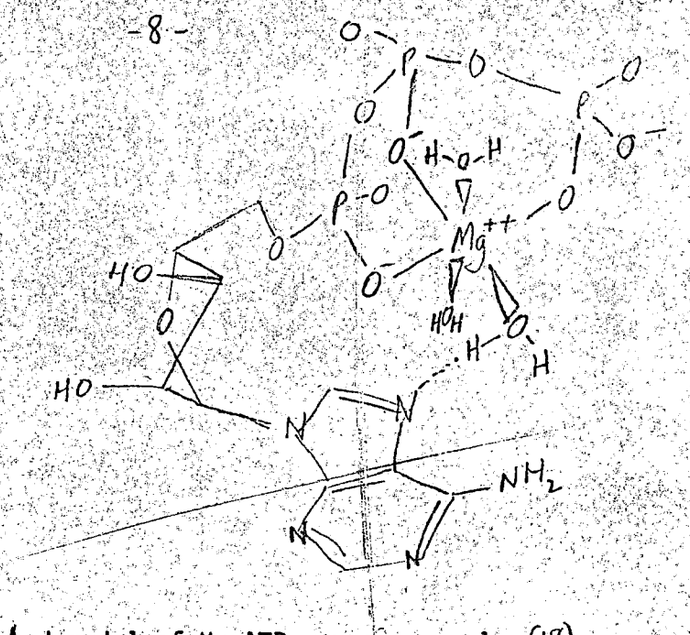

Figure II- Postulated model of Mg-ATP aqueous complex(18)

Metal	ATP	ADP	GTP	ITP	UTP	UDP	CTP	AMP
Ca^{2+}	3.77*	2.82	3.58	3.76	3.71	-	3.81	1.76
Mg^{2+}	4.04	3.15	4.02	4.04	4.02	3.17	4.01	1.95
Mn^{2+}	4.75	3.94	4.73	4.57	4.78	-	4.78	2.31
Co^{2+}	4.62	3.68	4.63	4.74	4.55	-	4.48	2.58

*Log K_f, u=0.1, pH=8.2, 23°C, 0.1M NaCl.

Figure III- Formation constants for the reaction:
metal ion + nucleotide $\rightleftharpoons^{Kf}$ complex

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THE RESONANCE THEORY AND ATP

While the pathways in which ATP is synthesized and those in which it is hydrolyzed are well-charted, the mechanism involving the transfer of energy either to ATP or from it is poorly understood. McClare (13) has proposed that the fundamental property of ATP is, that after hydrolysis, the internal energy change in the reaction first appears as a specific bond vibration. The enthalpy change in the hydrolysis of ATP is about 40 KJ/mole, which corresponds to an infra-red radiation with a frequency of 3,300 $cm.^{-1}$. This resonant energy could be trapped by a molecular oscillator acting as a receiver and thus converted to mechanical work. The synthesis of ATP on membrane-bound sites can also be explained: the resonance induced by electrons passing through transport chains in photophosphorylation and oxidative phosphorylation could provide the energy in the form of resonance for a "tuned-in" synthesis, in this case, of ATP.

Not all the ATP, however, is synthesized with energy from electron transport, and not all ATP hydrolysis is converted into mechanical work; thus the applicability of resonance to substrate-level phosphorylations and hydrolysis of ATP is less obvious. On the other hand, the rapidity of resonant energy transfer allows us to consider high efficiencies for molecular machines.

This theory has been presented here to the exclusion of numerous others because it has relevant implications. If the transfer of the phosphate bond to and from ATP involves resonant energy, then the specificity of the molecular participants becomes more important. The difference between other mononucleotide triphosphates and ATP may be postulated to extend beyond their degree of fitting into specific allosteric enzyme sites.

The various purine and pyrimidine bases all have different specific absorptions in infrared spectra. Some values of intermediate absorption peaks are presented in figure IV (14-17).

If this theory has some validity, then there also could exist a basis for the choice of ATP over GTP or UTP, on the basis of

Molecule	Peak (cm^{-1})	Type of absorption
Adenine	1665	strong
	1605	strong
	1573	weak
Uracil	1700	strong
	1680	strong
	1650	strong
	1620	weak
Thymine	1720	strong
	1660	weak
	1575	weak
Guanine	1690	strong
	1630	weak

Figure 4- Some absorption peaks in the infrared spectrum of various purine and pyrimidine bases (14-17). The individual spectra of adenine, uracil and guanine appear in ref. 19.

of resonance. The nucleotides may be just as similar in infrared absorption as they are in other physical and thermodynamic characteristics. When resonance is involved, however, a difference in an absorption part of a few cm^{-1} may make a big difference in energy-coupling and indeed serve as a criterion for selecting one molecule over a group of others.

HIGH-ENERGY PHOSPHATE COMPOUNDS IN THE "PRIMEVAL BROTH"

While no experiment to date has demonstrated that nucleotides or nucleosides can be formed from simple precursors under conditions which simulate those believed to have been present in prebiological times, different possible stages in their synthesis have been done.

The bases adenine, guanine and cytosine were among the products obtained with a reaction mixture containing CO_2, H_2 and NH_3, in a Fischer-Tropsch-like process, by Yang and Oro (19).

Ponnamperuma, Sagan, and Mariner (20), have succeeded in synthesizing ATP from ADP, AMP or adenosine in dilute aqueous solution, with ethyl metaphosphate and ultra-violet irradiation. In addition, the synthesis of AMP from adenosine has been accomplished (21), ~~from adenosine,~~ using cyanimide to activate orthophosphate.

Whether or not ATP was an actual component of the primeval organic soup is a moot question. However, one observation is interesting. In the experiments of Yang and Oro (19) mentioned above, quantitative analysis of results (from IR and UV spectra) showed that much more adenine was obtained than guanine or cytosine (Fig. 5). Moreover, in the absence of a catalyst, only adenine was found. Thus, even if nucleotides were not present in the primeval broth and the early cell had to synthesize them from base, sugar and phosphate (albeit, far different from present

BASE	Yield (based on conversion of NH_3)
Adenine	0.507%
Guanine	0.158%
Cytosine	0.127%

Fig. 5- Synthesis of adenine, guanine and cytosine by a Fishcer-Tropsch-like process. The reaction mixture contained CO_2, H_2, and NH_3 in a ratio of 1:2:0.4 (19).

enzymatic pathways in which the purine base is synthesized on the ribose-phosphate), the most available base may have been adenine and thus the prevalent triphosphate, ATP.

On the other hand, the presence of simpler molecules with "high-energy phosphate bonds" is much more likely. .Pyrophosphate has been synthesized under primitive earth conditions (21,22), and the ability of polyphosphate to act as donor of activated phosphate has been postulated (23). M. Baltscheffsky (7,24) showed that in at least one bacteria (Rhodospirillum rubrum), pyrophosphate can still function as a donor of biologically useful chemical energy . The possibility exists that this organism has either maintained or reverted back to an ancient method of energy-transfer which did not require adenosine phosphates (25). Polyphosphates are present in a variety of organisms (26) and could also have played a role in the evolution of phosphate metabolism.

The question remains: if there were other biologically- (or prebiologically-) active mediators of phosphate metabolism, what precipitated the transition to ATP, and what prevented further transitions from taking place throughout the evolutionary eons?

DISCUSSION-

> "ATP is a rather complicated molecule and while it is obviously well-suited to the needs of the organism, it is by no means unique."
>
> -E. Broda (27).

This statement is only partly correct. Perhaps ATP per se is not unique, but its biological role certainly appears to be. And if indeed ATP is not intrinsically unique, why has it been singled out in biochemical systems? Why has there been no successful search for a better substrate?

Many problems concerning energy metabolism have logical, plausible explanations. Phosphate has been shown to be the best moiety for the transfer of chemical energy (28). Silicates do not ionize sufficiently, sulfates are unstable in water, and arsenates are hydrolyzed non-specifically. The problem of dissolution of phosphate has been explained by the formation of calcium complexes with apatite (29) and organic complexing agents such as oxalate. Thus phosphate was perhaps selected over other ions because it possessed the best properties for energy storage.

When ATP is compared to other molecules with phosphate bonds, but not similar to it, a rationale also can be seen to exist for its selection. Its free energy of hydrolysis places it midway in the column of biological phosphate-containing molecules. The primary product of hydrolysis, ADP is a molecule very similar to it, which could constitute an advantage at the reaction site, and perhaps facilitate the reverse reaction of synthesis as well. Finally, when compared to a molecule such as pyrophosphate, or even phosphocreatine, the enzymatic recognition of a molecule such as ATP is much more specific. It can be bound at a number of sites and recognized at the exclusion of similar molecules (this general property of mono-

nucleotides undoubtedly explains their success as components of nucleic acids).

When the field is narrowed down to a comparison of ATP with molecules who differ in the base moiety, reasonable explanations become scarce. ATP and GTP are both one substitution away from ITP. All the mononucleotide triphosphates have similar physical characteristics. The equilibrium constant for the reaction

$$ATP + GDP = ADP + GTP$$

is one (28). And while UTP and UDP may not be quite as similar as ATP and ADP (28), the other mononucleotide phosphates can and do act as acceptors and donors of phosphate just like ATP, only on a much more limited scale, in biological systems. How did ATP "land" the star role?

The only reasonable hypothesis which could explain the prevalence of ATP in terms of its specific characteristics is the theory of resonant energy transfer. Much work remains to be done before this can be confirmed, and a specific vibration or group thereof can be ascribed to the reaction.

It could also be that pre-evolutionary biochemical history prescribed this preference for ATP. Perhaps adenine was the most common base and the synthesis of ATP preceded those of other mononucleotides. Perhaps the first phosphorylase had a coincidental higher affinity for ATP. Whether this primitive choice was random or not, once ensconced in primitive metabolism, it would become a difficult one to change.

Certain biological characteristics are beyond the grasp of Darwinian evolution. Proteins and the molecules they synthesize evolve, but the molecule of DNA (for example) is the same in every cell. The code it dictates is the same for E. Coli and for man (29). And its monomeric constituents have also remained the same. They cannot evolve because they make up the very molecule through which evolution is carried out. If the genetic code or the com-

ponents of DNA are modified, the transcription and translation processes are immediately curtailed and the cell cannot survive. Too many simultaneous mutations are required, and even so, there could be no phenotypic lag because the present apparatus could not succeed in synthesizing the necessary components to cope with the change.

This argument, however, does not preclude the possibility that in the course of primitive development of the cell, the genetic code did "evolve". Indications of such have been discussed (29,30,32,33). But, at a certain point in the gelling of the processes of transcription and translation, a turning point was reached, after which no further development of the basis of the genetic apparatus could be accomplished.

The same line of thought may be applied to ATP. Suppose, that the early phosphate-transferring metabolites were simple molecules such as pyrophosphate, but as biochemical processes in the protocell developed, ATP became the main-line phosphate mediator, for whatever reason. The enzymes involved in transferring energy to ATP and coupling its hydrolysis to other reactions developed specific sites with a high affinity for ATP to the exclusion of other molecules. At a certain point in primitive development, the cell became so dependent on ATP as an energy mediator that it could not evolve away from it. The living cell is dependent on a constant energy supply. A molecule of ATP turns over (in its terminal phosphate group) many times a second. During any attempt to replace ATP with another molecule, the cell would die first of energy starvation, not being able to immediately recognize and use another substrate.

The possibility of the preference for ATP involving a biological historical imperative, dating back to the primitive cell, leads us to a final speculation. Could the development of ATP as an energy source, and the development of mononucleotide triphosphates as precursors of nucleic acids have some historical connection?

Was ATP first synthesized as a precursor of DNA, and thereafter developed into an energy mediator? It is also a remote possibility that DNA originated as an intracellular polymer of monomers involved in energy metabolism, followed by its development into a genetic macromolecule.

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